Supplementary Information

Facile Synthesis of Tunable Zinc-Adenine Frameworks for Aptamer-Based Biological Applications

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1. Materials and methods

Materials

All chemicals were purchased from Sigma, unless otherwise stated. Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, Sigma Z4750), adenine ($C_5H_5N_5$, Alfa Aesar A14906), HEPES ($C_8H_{18}N_2O_4S$, Acros Organics 172570250) were purchased from commercial suppliers at an analytical grade. Milli-Q water (≥18 MΩ·cm) or nuclease-free water (Integrated DNA Technologies) was used for all reagent preparations. Aptamers are purchased from Integrated DNA Technologies. The detailed sequence of oligos were provided in **Supplemental table S1**.

Blood collection

Blood products were obtained from healthy volunteers with informed consents under the guidelines of human ethics approved by Griffith University human ethics committee (approval number: 2021/598). A total of 10 mL of citrated blood was collected from a healthy donor (male, 32 years old) and mixed with 250 µL of 1 M $CaCl_2$ (prepared in 0.9% NaCl) to induce coagulation at room temperature for 1 h. The sample was then centrifuged at 2,000×g for 30 min at 4°C, and the supernatant was collected as healthy serum.

Particle synthesis

Typically, 45 µL of $ZnSO_4$ (100 mM) was mixed into 955 µL HEPES buffer (100 mM, pH 7.4) containing 1.5 mM adenine and 50 nM aptamer (if used), using a thermoshaker (1200 rpm, room temperature, 10 min). The precipitates were collected and washed 3 times with mili-Q water by centrifugation at 10,000 ×g for 5 min. Theoretically, one reaction yields approximately 0.4 mg of ZnMOF material under these conditions. The collected native ZnMOF was incubated in poly(sodium 4-styrenesulfonate) (PSS, 1 mg/mL) for 10 min in most experiments. Sonication, if used, was carried out in a bath sonicator for 5 min. The final precipitates were collected by centrifugation at max speed (17,000 ×g) for 5 min for particle characterization.

Stoichiometry analysis

For stoichiometry analysis, different concentrations of $ZnSO_4$ were mixed with 1.5 mM of adenine in HEPES buffer (100mM, pH 7.4). After centrifugation at 10,000 ×g for 5 min, the quantity of unreacted adenine (in supernatant fraction) was assessed by absorbance 260 nm reading via a NanoDrop ND-1000 UV-Vis Spectrophotometer (Nanodrop Technologies, Wilmington, DE), following an adenine calibration curve (**Supplemental figure S2**).

Particle characterisation

The hydrodynamic size of the particles was examined using dynamic light scattering (DLS) mode on an Anton Paar LiteSizer 500 machine. To determine the particle size, 1 mL of particles were suspended in a disposable cuvette (10x10x45 mm). One milliliter of the same mixture was measured in an omega cuvette utilizing the Zeta measurement mode for the Zeta potential analysis.

Transmission electron microscopy

The samples were drop-mounted onto Formvar/carbon-coated 200 mesh copper grids (for 2-3 min), the excess water was removed. Once air-dried, the sample images were collected on a JEM-1010 Transmission electron microscope (JEOL Ltd.) at Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland (St Lucia, Brisbane), operating at an accelerating voltage of 120 kV.

Immunofluorescent assay with hypoxia culture

SVEC4-10 endothelial cells were seeded overnight in a 12-well plate (100,000 cells/well). After changing media, cells were cultured either in normoxic or hypoxic (0.1% O_2, 5% CO_2) condition or stimulated with lipopolysaccharide (LPS, 5 μg/mL) for 24 hr. Cell was stained for 1 h with naked VCAM-1 aptamer (100 nM) or VCAM-1 aptamer@ZnMOF (calibrated for equivalent amount of aptamer). After washing, an epi-fluorescent microscope was used for imaging (40X objective lense, Olympus). Sapphire FL Biomolecular Imager (Azure Biosystems, CA, USA) was used for whole-well fluorescent imaging. Qualification was performed with ImageJ software.

Flow cytometry

SVEC4-10 endothelial cells were seeded in 6-well plates at a density of 200,000 cells per well and cultured overnight in DMEM supplemented with 10% FBS. Cells were then subjected to normoxia, hypoxia (0.1% O_2, 5% CO_2) or stimulated with lipopolysaccharide (LPS, 5 μg/mL) for 24 hr. Following treatment, cells were washed twice with cold PBS, fixed with CytoFlix fixation buffer at 4^{o}C for 30 min, then incubated for 1 h at room temperature with PBS buffer containing 1% BSA with either naked VCAM-1 aptamer (100 nM) or VCAM-1 aptamer@ZnMOF (normalized to an equivalent aptamer concentration). Flow cytometry was performed on CyFlow Cube 6 (Sysmex Corporation) equipped with a 488 nm laser for FITC detection. A minimum of 10,000 events were collected per sample.

Cell culture and preparation of input exosome

SVEC4-10 endothelial cells (ATCC CRL-2181) were cultured in DMEM medium (ATCC 30-2002) supplemented with 10% fetal bovine serum (FBS, Gibco A3160902) until 60% confluent. Culture medium was replaced with media containing 10% exosome-depleted FBS (Gibco A2720801) and cells were grown for another 24 hours. After that, culture media from several flasks were collected and pooled together, filtered, and ultracentrifuged. A volume of 50 mL of cell culture media was 0.22 μm-filtered and ultracentrifuged at 100,000 ×g for 120 min. The resulting exosome pellet (typically not visually apparent) was resuspended in 1 mL of cell culture media and considered as the input for exosome isolation.

Exosome isolation with CD63 aptamer@ZnMOF

After PSS treatment (described in Particle synthesis section), 0.4 mg pellet of CD63 aptamer@ZnMOF was collected and resuspended in 500 μL of input exosome and incubated for 4 h with rocking. After centrifugation at 10,000 × g for 5 min, the supernatant containing the unbound exosome was collected for nanoparticle tracking analysis to estimate the EVs capture efficiency. The pellets containing exosome-binding CD63 aptamer@ZnMOF were

incubated with RVseq oligo (5 μM in PBS buffer, 30 min) for the release of exosome. After centrifugation, the supernatant containing rescued exosome was collected for nanoparticle tracking analysis to estimate whole process recovery.

Nanoparticle Tracking Analysis

Nanoparticle tracking analysis (NTA) was carried out with a NanoSight NS300 system (Malvern, PA, USA). The samples were diluted with PBS (if necessary) and subsequently introduced into the instrument using the following script: PUMPLOAD, REPEATSTART, PRIME, DELAY 10, CAPTURE 30, and REPEAT 5. Videos were recorded at a camera level of 10, a camera shutter speed of 20 ms and a camera gain of 600; these settings were kept constant between samples. Each video was subsequently analyzed to determine the concentration, mean, mode, and size distribution of exosomes.

Western blot analysis

Cell lysate or enriched exosomes were denatured in 5× sample buffer and heated at 95°C for 5 min. 15 μL of the lysed samples were resolved by 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes by transblotting (Bio-Rad, Hercules, CA, USA). The PVDF membrane was incubated in blocking buffer (3% BSA in TBS-T) for 1 h at room temperature and then incubated with either CD9 antibody (10626D, Invitrogen, MA, USA) (1:1,000), CD63 antibody (10628D, Invitrogen) (1:1,000), Cytochrome c antibody (7H8.2C12, Invitrogen) (1:1000), or Calnexin antibody (GT1563, Invitrogen) (1:1000) . After washing, the membrane was incubated with an HRP-conjugated goat anti-rabbit secondary antibody (1706515; Bio-Rad) (1:4,000; diluted in blocking buffer) for 2 h at room temperature. Detection was carried out with SuperSignal West Pico Chemiluminescent Substrate kit (Thermo Scientific) following supplier's manual instructions.

Loop-mediated isothermal amplification (LAMP) of exosomal mRNA

Cell culture supernatant was collected, centrifuged and 0.22 μm-filtered to remove cells and debris. 1 mL of cell culture supernatant, without any further concentration, was directly resuspended into 0.4 mg CD63 aptamer@ZnMOF pellet and incubated for 4 h at room temperature with rocking. After centrifugation at 10,000 × g for 5 min, the pellet containing the exosome-bound ZnMOF was collected and incubated with RVseq oligo (5 μM in PBS buffer) for the release of exosome. After centrifugation, the supernatant containing rescued exosome was collected and heated at 95°C for 5 min to induce the release of nucleic acid contents.[1] The resulting exosomal lysate can be subjected to LAMP-based isothermal amplification.

Reverse Transcriptase Loop-mediated isothermal amplification was performed with WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix with UDG (E1708, New England Biolabs, MA, USA). All LAMP primers were purchased at a concentration of 100 μM. To reduce pipetting variability, a 10× primer mix stock (16 μM of inner primers and 2 μM of outer primers) was prepared according to NEB suggested protocol. Detailed sequences of primers were provided in **Supplemental table S1**. To reduce pipetting variability, a 10X primer mix stock was prepared according to NEB suggested protocol.

For each reaction, 2µL of RNA template sample was used and the volume was adjusted with nuclease-free water to 25 µL, following manufacturer's recommended concentration of master mix, primer set and fluorescent dye. Per 25 µL reaction, the following were added: 12.5 µL Master mix, 2.5 µL primer mix, 0.5 µL fluorescence dye, 2 µL RNA sample, 7.5 µL nuclease-free water. Reaction program was set up at 60°C so that fluorescence reading was carried out for approximately every 60 s (including ~7 s for plate reading time) in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). A no-template control (NTC) sample using nuclease-free water instead of RNA template sample was always included.

Synthetic ALPL mRNA was synthesized from gBlock gene fragments (Integrated DNA Technologies) using a HiScribe™ T7 Quick High Yield RNA Synthesis Kit (E2050, NEB, MA, USA) following the manufacturer's instructions. Briefly, a 30 µL transcription reaction containing 1 µg of DNA template, T7 RNA Polymerase Mix and NTP buffer mix (6.7 mM of each NTP final) was incubated at 37°C for 4 h. To remove template DNA, 30 µL of nuclease-free water and 2 µL of DNase I was added and incubated at 37°C for 15 min. The synthesized RNA was then purified using LiCl precipitation procedure provided by NEB. Calibration curve for qRT-LAMP was then constructed with synthetic mRNA target (**Supplemental figure S3**).

Density Functional Theory (DFT) calculations

We performed Density Functional Theory (DFT) calculation to study the structure of the as-synthesized ZnMOF material and the binding of aptamer on it. All the DFT calculations were performed using the Vienna ab-initio simulation program (VASP) package,[2, 3] utilizing spin-polarized setting, cut-off energy of 520 eV, Perdew-Burke-Ernzerhof (PBE) exchange-correlation functional,[4] and Gaussian smearing method with smearing width of 0.01 eV. All the structural optimizations were done using the conjugate-gradient method in the 20Å × 20Å × 20Å simulation unit cell and the Brillouin zones were sampled by Γ-centered k-point grid densities.

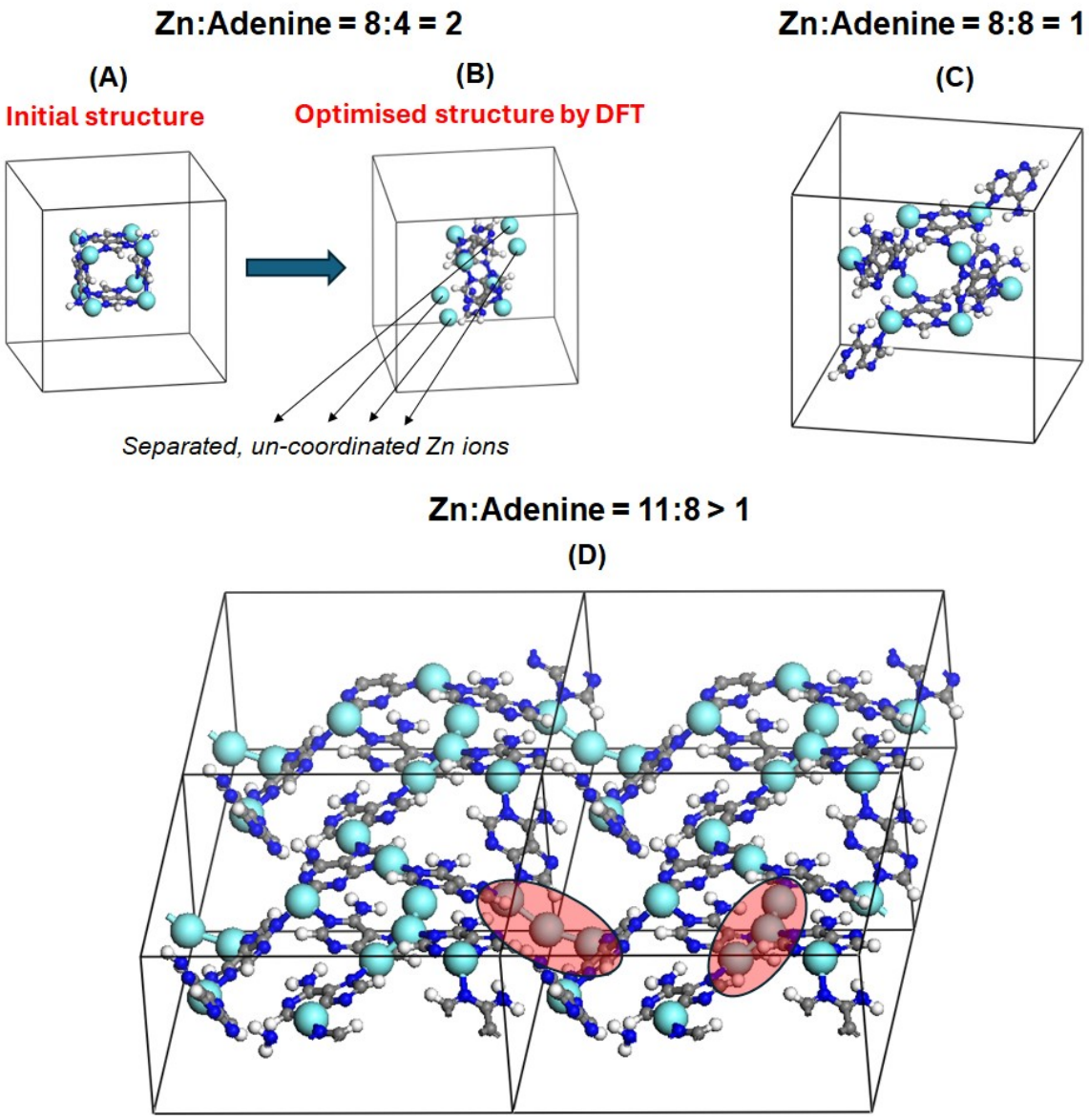
Statistical analysis

Data are presented as means ± standard deviation of triplicate. Graphical representations of the data were constructed using GraphPad Prism software (GraphPad Software Inc., CA, USA). Student's *t*-test were employed for significance testing with a p-value ≤ 0.05 considered statistically significant.

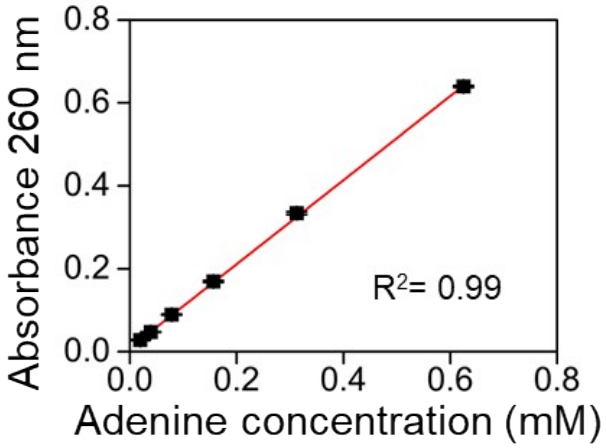
2. Supplementary table and figures

Supplemental table S1: Oligonucleotide sequences used in this study

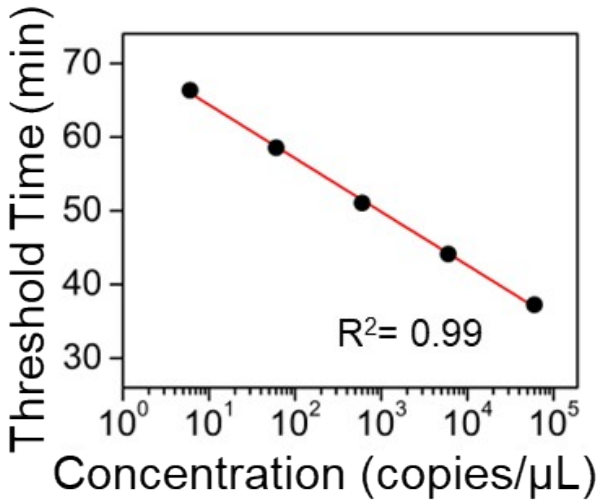
Oligo	Sequences
VCAM-1 aptamer	ATA CCA GCT TAT TCA ATT GGA CAC GGC AAA GGG GTA TAG CCT ACC GGA CCG TGA ACA TGG AAT GGT GTG CTG CGT GGA GAT AGT AAG TGC AAT CTA AAA A.
CD63 aptamer	CAC CCC ACC TCG CTC CCG TGA CAC TAA TGC TAA AAA AAA AAA AAA.
Scramble aptamer	CGA CCG GTC TGG GGA CCC TGT CTG GGT GTG TGG GTA GTA GGT CGA AAA
RVseq oligo	TTT TTT TTT TTT TTA GCA TTA GTG TCA CGG GAG CGA GGT GGG GTG
F3 primer	TGG CCC TCT CCA AGA CAT
B3 primer	GGA CTT CCC AGC ATC CTT G
FIP primer	TCA CGC CAC ACA AGT AGG CAG ATA ACA CCA ACG CTC AGG TC
BIP primer	GAG TGA GCG CAG CCA CAG AGC GCA GGA TGG ATG TGA CC



Supplemental Figure S1. Optimized structures from DFT calculations for ZnMOF materials at different Zn:Adenine atomic ratios. To investigate the structure of ZnMOF, we started with the model of Zn:Adenine = 8:4 = 2 (**Figure S3A**) to match the titration results of Zn:Adenine atomic ratio = 2 in **Figure 1D** of the main text. However, upon DFT optimization, 4 Zn atoms are separated and could not coordinate with the adenine molecules (**Figure S3B**). We increased the number of adenine molecules to match the Zn:Adenine atomic ratio = 8:8 and obtained the well coordination between Zn atoms and the adenine (**Figure S3C**). However, the terminal adenine molecules in this structure (**Fig. S3C**) could not form periodic structure via cross-linking with the adjacent structures in repeated unit cells and therefore the ZnMOF could not be crystallized. We further adjusted the Zn:Adenine atomic ratio = 11:8, and DFT optimization showed the formation of some Zn clusters at this atomic ratio (**Figure S3D**). In this structure, the periodic structure of ZnMOF spanning repeated unit cell is observed, suggesting that the crystallization of the ZnMOF is feasible. Therefore, it is likely that the formation of Zn cluster at Zn:Adenine atomic ratio larger than 1 caused the distortion of the orientation of adenine molecules, resulting in the formation of amorphous structure for the ZnMOF material.



Supplemental Figure S2. Adenine calibration curve with Absorbance 260 nm measurement.



Supplemental Figure S3. Calibration curve for qRT-LAMP using synthetic ALPL mRNA

3. References

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